Title: Phenotypic plasticity and genetic adaptation of functional traits influences intra-specific variation in hydraulic efficiency and safety

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Abstract:

Understanding which hydraulic traits are under genetic control and/or are phenotypically plastic is essential in understanding how tree species will respond to rapid shifts in climate. We quantified hydraulic traits in *Eucalyptus obliqua* across a precipitation gradient in the field to describe: 1) trait variation in relation to long-term climate and 2) the short-term (seasonal) ability of traits to adjust (i.e., phenotypic plasticity). Seedlings from each field population were raised under controlled conditions to assess: 3) which traits are under strong genetic control. In the field, drier populations had smaller leaves with anatomically thicker xylem vessel walls, a lower leaf hydraulic vulnerability and a lower water potential at turgor loss point, which likely confers higher hydraulic safety. Traits such as the water potential at turgor loss point and ratio of sapwood to leaf area (Huber Value) showed significant adjustment from wet to dry conditions in the field, indicating phenotypic plasticity and importantly, the ability to increase hydraulic safety in the short-term. In the nursery, seedlings from drier populations had smaller leaves and a lower leaf hydraulic vulnerability, suggesting key traits associated with hydraulic safety are under strong genetic control. Overall, our study suggests that strong genetic control over traits associated with hydraulic safety, which may compromise the survival of wet-origin populations in drier future climates. However, phenotypic plasticity in physiological and morphological traits may confer sufficient hydraulic safety to facilitate genetic adaptation.

Introduction

The frequency and intensity of droughts are predicted to increase in the future (IPCC, 2013), and trees around the world are thus likely to experience increased drought stress (Allen *et al.*, 2010; Williams *et al.*, 2012). Hence, there is a need to understand the factors or traits that define drought resistance in trees. Various functional traits have emerged as useful indicators of tree drought resistance, such as vulnerability to embolism (Choat *et al.*, 2012), turgor loss point (Bartlett *et al.*, 2012), hydraulic safety margin (Anderegg *et al.*, 2016), leaf morphology (Yates *et al.*, 2010), leaf anatomy (Scoffoni *et al.*, 2017), and leaf vein architecture (Sack & Scoffoni, 2013). Most studies, however, focus on a few drought resistance traits, limiting our understanding of the complex contributions various plant hydraulic traits have to drought resistance. To investigate drought resistance in greater detail, a multivariate approach which includes hydraulic and structural traits is needed (Anderegg, 2015).

Functional traits often change along environmental gradients (Warren *et al.*, 2006; Carter & White, 2009; Pfautsch *et al.*, 2016), as trees trade-off efficiency and persistence (Givnish, 1987; Reich & Cornelissen, 2014; Stuart-Haëntjens *et al.*, 2018). Applying an economic approach to plant hydraulics, we would expect different hydraulic strategies of trees along environmental gradients depending on the water availability within the environment (Gleason *et al.*, 2016). Indeed, compared with trees from wetter climates, dry-origin trees have been reported to have smaller xylem vessel diameters (Pfautsch *et al.*, 2016; Schuldt *et al.*, 2016), a lower xylem vulnerability to embolism (Scholz *et al.*, 2013), thicker pit membranes (Sperry *et al.*, 2006; Schuldt *et al.*, 2016), a lower turgor loss point (Ψ_{TLP}) (Bartlett *et al.*, 2012), smaller leaves (Nardini *et al.*, 2014), a lower specific leaf area (SLA) (Warren *et al.*, 2005) and Huber Value (HV) (Carter & White, 2009). Hence, these trait differences provide the level of hydraulic safety or efficiency species require to survive their home environment. Thus, functional traits can be used as a proxy to describe the hydraulic trade-off between efficiency and safety, arising primarily through water limitation in the environment (Table **1**). Here, we define *hydraulic efficiency* as measures which increase the rate or amount of water transport across a given pressure gradient and *hydraulic safety* as measures which reduce the cavitation risk of the conducting tissue. An example of a trait conferring *hydraulic efficiency* is the vessel diameter of xylem conduits which strongly influences the velocity of water transport, as described by the Hagen–Poiseuille equation (Tyree & Zimmermann, 2002). A trait closely linked to *hydraulic safety* is the xylem

vulnerability to cavitation, which is associated with water potential thresholds that result in drought-induced tree mortality (Nardini *et al.*, 2013).

While most current research focusses on understanding inter-specific differences in tree functional traits and the implications for drought resistance (Anderegg *et al.*, 2016; Choat *et al.*, 2018; Johnson *et al.*, 2018; Li *et al.*, 2018), there is comparatively little research on intra-specific differences in tree drought resistance (Nicotra *et al.*, 2010). Studies on trees (Bresson *et al.*, 2011; Rosas *et al.*, 2019), shrubs (Jiang *et al.*, 2016) and grasses (Harzé *et al.*, 2016) indicate large intra-specific variation in functional traits, resulting in diverse drought resistances even within a species (Bréda *et al.*, 2006; Choat *et al.*, 2007). Within a given species, dry-origin populations also tend to show smaller xylem vessel diameters, smaller leaves and a lower SLA (Bresson *et al.*, 2011; Corcuera *et al.*, 2012; McLean *et al.*, 2014; Peguero-Pina *et al.*, 2014; Knutzen *et al.*, 2015; Hajek *et al.*, 2016; Ramírez-Valiente *et al.*, 2017). Intra-specific studies examining the sapwood vulnerability to embolism are inconclusive, with some studies observing a lower vulnerability to embolism in dry-origin populations (Corcuera *et al.*, 2011; Lamy *et al.*, 2014; Peguero-Pina *et al.*, 2014; Stojnić *et al.*, 2018), while Maherali and DeLucia (2000) and Lobo *et al.* (2018) observed no difference among populations. Nevertheless, intra-specific differences in the sapwood vulnerability to embolism seem, especially in gymnosperms, to be limited (Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2014). The only study which measured intra-specific differences in leaf hydraulic vulnerability came to similar conclusions (Lucani *et al.*, 2019a). However, the two *Eucalyptus globulus* population studied by Lucani *et al.* (2019a) occurred both under mesic environmental conditions and therefore might not naturally select for a reduced leaf hydraulic vulnerability. Hence, despite the importance of leaf function for gas-exchange (Brodribb *et al.*, 2005), we lack the knowledge if leaf hydraulic vulnerability is under genetic control and if so, whether intra-specific genetic diversity is limited to a similar degree as observed for sapwood vulnerability.

Despite the importance of intra-specific trait variability for species survival and demography under climate change, we know little about the individual contribution of adaptive genetic differences and phenotypic plasticity (Drake *et al.*, 2015; Benomar *et al.*, 2016), the main processes influencing plant adaptation (Mitchell & Bakker, 2014). The genetic adaptation of trees to their environment and their longevity are of major concern in the context of climate change (Bréda *et al.*, 2006), as trees will need to adapt or adjust rapidly to prevent local extinction (Aitken *et al.*, 2008). To enable rapid adaptation, a high genetic

diversity within and between populations is necessary, as a high genetic diversity improves the chances for a genetic variation to occur which improves the species' drought resistance (Jump *et al.*, 2009). Hence, determining which drought resistance traits are under genetic control will improve our understanding of the vulnerability of species towards drought. Recent studies have shown intra-specific genotypic variation in physiological (Knutzen *et al.*, 2015), morphological (Benomar *et al.*, 2015; Schuldt *et al.*, 2016) and xylem anatomical traits (Hajek *et al.*, 2016). Traits under strong genetic control may pose a challenge for plants to survive in a rapidly drying environment as traits are unable to adjust rapidly to new environmental conditions. On the other hand, traits that are phenotypically plastic can potentially compensate for mismatches between actual environmental conditions and the conditions to which the genetically controlled traits are adapted (Nicotra *et al.*, 2010). Studies have shown that leaf morphological, physiological (Pita & Pardos, 2001; Corcuera *et al.*, 2011; McLean *et al.*, 2014; Johnson *et al.*, 2018) and anatomical traits (Mitchell & Worledge, 2015) are phenotypically plastic and can adjust to different environmental conditions. However, there are only a few studies that investigated comprehensively which anatomical, morphological and physiological plant hydraulic traits are under genetic control or are phenotypically plastic among different populations of a single tree species.

Here, we evaluate intra-specific variation in hydraulic trait expression in *Eucalyptus obliqua* L'Hér. growing across a precipitation gradient with mean annual precipitation (MAP) ranging between 600 and 1000 mm. We quantified hydraulic traits in the field to describe: 1) trait variation across the precipitation gradient and 2) short-term (seasonal) trait adjustment (i.e., phenotypic plasticity). Seedlings from each population sampled in the field were also raised under controlled conditions to assess: 3) which traits are under strong genetic control. We hypothesised that anatomical and morphological traits would be under strong genetic control, whereas physiological traits would show phenotypic plasticity. We discuss the relative importance of genetic control and phenotypic plasticity for key traits in the context of hydraulic safety and efficiency.

Material and Methods

Eucalyptus obliqua field sites

Eucalyptus obliqua is an evergreen, widespread eucalypt which occurs naturally in southeast Australia across a wide mean annual precipitation (MAP) gradient, ranging from

500 to 2400 mm yr^{-1} (Green, 1971; Boland *et al.*, 1992). We selected five field sites along a climate gradient, with the most southern site, Brisbane Ranges (BR), having the highest mean annual temperature (MAT) and lowest MAP (638 mm). To the north, the Central Victorian Uplands increases in elevation, resulting in colder and wetter conditions. The wettest and coldest site on the MAP gradient is Leonards Hill (LH) with a MAT of 10.4°C and 60% greater precipitation than the BR site (Table **2**). LH climatically reflects the species' distribution centre, while BR represents the warmer and drier edge of the distribution (Green, 1971). *E. obliqua* at all sites occurred in even-aged (7 - 15 years) regrowth stands. Species composition consisted of ≥ 80% cover of *E. obliqua* on all sites, except for BR which had only 20% cover.

The MAP and MAT for all sites were obtained through the climate layer available in the Atlas of Living Australia which uses ANUCLIM v6 (beta) with 0.01 degree resolution (~1km) and centred on 1990 (Williams *et al.*, 2010). The Heat Moisture Index (HMI) was calculated to account for the combined influence of the precipitation- and temperature gradient on trait expression. HMI was calculated using the formula (Wang *et al.*, 2006):

$$HMI = \frac{MAT + 10}{\frac{MAP}{1000}} \quad \text{(Eq.1)}$$

Temperature, HMI and elevation were highly correlated with precipitation across the field sites (Fig. **S1**).

Nursery experiment

The nursery experiment was located at the Burnley campus of the University of Melbourne, Australia. The MAT at the site was 14.8°C which was at least 1.8°C warmer than those experienced by any wild population. We collected seeds from five to eight individuals per population in a 200 m radius around the GPS location (Table **2**) and germinated them in September 2016 in plastic seedling trays with a mixture of pine bark, fine sand, sieved coir, peat, and dolomite. Trays were first placed in a glasshouse to germinate and establish. After two months, saplings were planted into 15 litre pots and placed outside where they were watered every day. Wind and animal damage resulted in a loss of some individuals, with only five trees remaining from the DF population (seed origin at 822 MAP) and eight to 10 trees for all other populations.

Functional trait sampling design

We conducted physiological, morphological and leaf xylem anatomical measurements at each field site during the dry summer (February 2016) and wet winter (June 2016) season. Here, seasonal shifts in trait expression were assumed to represent phenotypic plasticity in response to water availability (Chevin *et al.*, 2010). Our comparison of the magnitude of phenotypic plasticity among populations is therefore not independent of different levels of water availability across the rainfall gradient. From each field population, two branches (branch diameter of ~1 cm) from the sun-exposed canopy of 10 trees were randomly collected, double bagged, and transported to the laboratory at the University of Melbourne for all functional trait measurements (except for pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) water potentials). Functional trait measurements on the 8-month-old saplings in the nursery were performed only during the wet season (June 2017). All measurements (Table **1**) were performed on five to 10 trees per population and if not otherwise specified, the same methods were applied for measuring the functional traits in the field and in the nursery experiment.

Physiological trait measurements

We measured seasonal changes in Ψ_{PD} and Ψ_{MD} (only in the field) and performed pressure-volume curves as well as leaf xylem vulnerability curves to evaluate tree physiology. Pre-dawn and midday water potentials were measured for two leaves per tree, on at least six trees per site during the wet and dry season using a pressure chamber (Model 3000, Soilmoisture Equipment Cooperation, Santa Barbara, CA, USA). A minimum of five leaves (one leaf per tree) per population was used to perform pressure-volume curves using the bench drying method, following the description given by Sanders and Arndt (2012). Leaves were rehydrated for at least two hours to full saturation before the start of the measurements. The Ψ_{TLP} was derived from the curve of $1/\Psi$ against relative water content using a fitting routine available online (http://landflux.org) based on Schulte and Hinckley (1985).

Leaf hydraulic vulnerability was measured following the method described in Melcher *et al.* (2012). To generate a vulnerability curve, we conducted repeated measurements of hydraulic conductance and the corresponding water potentials while the branches was drying in the laboratory. Leaf hydraulic conductance (K_{leaf}) was determined by measuring the 'initial' water potential of a leaf, while using the neighbouring leaf to measure the hydraulic flux during leaf rehydration (Brodribb & Cochard, 2009). A flowmeter with a calibrated resistor was used to determine the hydraulic flux, (F, mmol s^{-1}) of the

neighbouring leaf which was cut under water and directly attached for a minimum of 20 seconds to the flowmeter. A pressure chamber and leaf area meter (Li-Cor Li 3100, Li-Cor Inc., Lincoln, NE, USA) were used to determine the 'final' leaf water potential (MPa) and leaf area (m^2) after detaching the leaves from the flowmeter. Leaf hydraulic conductance, (K_{leaf}, mmol m^{-2} s^{-1} MPa^{-1}) was calculated using the following equation:

$$K_{leaf} = (F / \Psi) / LA \quad \text{(Eq.2)}$$

where Ψ can either be the 'initial' Ψ combined with F measured after attaching the leaf to the flow meter or it can be the 'final' Ψ combined with F measured before detaching the leaf from the flowmeter. If there was a large variation between the two obtained K_{leaf} values (percentage error ((initial K_{leaf} - final K_{leaf}) / initial K_{leaf})*100) > 15%), the measurement was discarded, otherwise both values were averaged, and the mean conductance used. A Weibull function from the R package *fitplc* (Duursma & Choat, 2017) was used to describe the relationship between K_{leaf} and initial Ψ and the loss of 50% (P_{50}) and 88% (P_{88}) conductivity were derived. The maximum conductivity (K_{max}) was derived by averaging K_{leaf} over at least 3 measurements at leaf water potentials > -1 MPa. All established leaf vulnerability curves are displayed in the supplementary material (see Fig. **S2** available as Supplementary Data at Tree Physiology Online). Further, the interaction between K_{max} and P_{50} was assessed in the field as well as in the nursery (Fig. **S3**). To assess the risk of hydraulic failure at each site, we calculated the hydraulic safety margin for each *E. obliqua* population as the difference between summer midday water potential and P_{50} and P_{88} (Choat *et al.*, 2012).

Morphological trait measurements

Morphological traits included: leaf size (LA), specific leaf area (SLA) and Huber Value (HV, sapwood area / leaf area). The HV (Tyree & Zimmermann, 2002) was measured on five twigs (branch diameter of ~ 0.3 cm) per tree. Sapwood area of the twig was measured using the image analysis software ImageJ (Schneider *et al.*, 2012) and cumulative leaf area was measured with a leaf area meter. The LA was derived by dividing the cumulative leaf area by the number of leaves per individual twig and SLA represented the twig leaf area divided by the leaf dry weight. The dry weight was recorded after oven drying leaves for 48 h at 65°C. The LA and SLA were measured in the nursery saplings as outlined above, but HV was derived from the sapwood area at the stem base divided by the total leaf area of the plant.

Leaf xylem anatomical trait measurements

The first fully expanded leaf from a sun-exposed twig was selected during the wet season in 2016 to measure leaf xylem anatomical properties. Leaf samples were dehydrated for 2 days each in an ethanol dilution series (25%, 50%, 70%, 100% ethanol). Samples were infused with LR White acrylic resin (No. 62661, Sigma Aldrich Pty Ltd, MO, USA) using an LR white / ethanol dilution series (50%, 70%, 100%; 2d in each) and polymerised at 65°C for 1d before sectioning. Sections (5 µm) were cut with an automatic microtome (1140 / autocut, Reichert-Jung, Heidelberg, Germany), stained with 1% Safranin solution and mounted in Entellan synthetic resin (Merck, Darmstadt, Germany). Micrographs of cross-sections were taken at ×100 and ×400 magnifications (Nikon Eclipse E 300 mounted with a Nikon Coolpix 995 camera). Anatomical characteristics were measured with the commercial software WinCELL (version 2016c, Regent Instruments Inc., Canada). The leaf xylem anatomy was studied in a 0.01 – 0.03 mm^2 wide section located in the first ¼ of the leaf, *e.g.*, furthest from the leaf tip where the midrib was the widest. A total of 50 to 250 individual secondary xylem vessels per leaf were measured over the whole xylem length to obtain a good representation of the various size classes. The vessel characteristics measured, were the radial vessel wall thickness (µm) and the radial lumen vessel diameter (µm), which were then used to calculate the double wall thickness of adjacent vessels (Wall, µm) and the hydraulic weighted vessel diameter (D_h) following (Tyree & Zimmermann, 2002):

$$D_h = \frac{[\sum D^4]^{0.25}}{N} \qquad \text{(Eq.3)}$$

where D is the vessel diameter and N the number of vessels. In the field, eight to 10 trees per population were used to derive the leaf xylem anatomical traits, whereas in the nursery experiment five to eight trees per population was used.

Statistical analysis

All statistical analyses were performed using R (version 3.4.3, R Core Team, 2017). To address research question one (do traits vary with MAP at origin), we fitted generalized linear mixed models (GLMM, package: *nlme*) for each functional trait with MAP, season and the interaction (MAP x season) as fixed factors. We used a nested site and tree within site random effect structure to account for repeated measurements per tree and differences in site conditions and a Gamma error distribution to account for heteroskedasticity. The models for Ψ_{TLP}, D_h and K_{max} were fitted with site as the only random factor, as there was only one

measurement per tree/ population. We normalized MAP predictor around the DF population (mean = 0, SD = 1) and all explanatory variables were log-transformed. To address research question two (is seasonal phenotypic plasticity expressed in the traits) we used significance testing of season and the interaction between MAP and season. A significant seasonal effect indicated phenotypic plasticity between the seasons, whereas a significant MAP x Season interaction indicated differences in the phenotypic plasticity among populations for that trait. To address research question three (does intra-specific genetic differentiation influence trait expression) we used the same approach as described for the field, *i.e.*, we used the nursery dataset and generated GLMMs for each trait. We used a 'tree' random effect for all functional traits with multiple measurements per tree, a Gamma error distribution to account for heteroskedasticity and log-transformed all traits. Significant population differences were tested through Tukeys post-hoc test ($P < 0.05$) to indicate genetic differentiation between the *E. obliqua* populations. We further used GLMMs to test if the observed trait differences were a result of adaptive genetic adaptation to the seed origin MAP.

Results

Trait variation along the precipitation gradient

To determine whether water relations traits changed along a natural precipitation gradient, we analysed wet season functional trait expressions for each population (Fig.**1**). In general, populations from drier sites were characterised during the wet season by a lower Ψ_{TLP}, higher Ψ_{MD}, smaller LA and lower $P_{50/88}$ (Table **3**). We did not observe significant functional trait changes for the wet season expression of Ψ_{PD}, HV, SLA, K_{max}, D_h or Wall (Fig. **1**). The driest population had the lowest leaf hydraulic vulnerability (BR; P_{50} = -3.69 MPa) and the wettest site had the highest vulnerability (LH; P_{50} = -2.76 MPa). Field populations with lower P_{50} also tended to have higher K_{max} (Fig. **S3**). Furthermore, leaf size doubled along the precipitation gradient from 13.3 cm^2 per leaf at the driest site (BR) to 28.1 cm^2 per leaf at the wettest site (LH). Values of Ψ_{TLP} ranged between -1.9 MPa at the driest and -1.77 MPa at the wettest site. Overall, there were substantial differences in trait expression among *E. obliqua* populations.

Short-term trait adjustment - seasonal trait variation

We further examined whether seasonal trait adjustment occurs by comparing dry (summer) and wet (winter) season trait expression, and whether the magnitude of plastic

adjustment was uniform across the populations. Apart from LA, all traits measured in both seasons differed in their trait expression seasonally (Table **3**). During the wet season all populations remained well hydrated (Ψ_{PD} ~ -0.15 MPa), whereas at the end of the dry season Ψ_{PD} decreased across all populations to -1.21 MPa, indicating water deficit at all sites, with the driest site (BR) having the most negative Ψ_{PD}. Differences in drought stress among the field sites (as indicated by different Ψ_{PD} in the dry season) can generate different magnitudes of phenotypic plasticity. The interaction term 'MAP x Season' used in the GLMM indicates whether differences in the magnitude of plasticity exist among populations but cannot indicate whether this magnitude would change if all populations were exposed to the same level of drought stress. A significant correlation of the interaction term (MAP x season; Table **3**), indicated differences in phenotypic plasticity among populations for Ψ_{MD} and SLA. *E. obliqua* populations from drier sites had a greater magnitude of phenotypic plasticity in Ψ_{MD} than populations from wetter sites (Fig. **1b**). Interestingly, SLA in the wet season was uniform across all populations, whereas at the end of the dry season, trees from the dry population had lower SLA than trees from the wet populations and the DF populations (~ 800 mm MAP) changed the least. The adjustment in Ψ_{TLP} from the wet to dry season was characterised by a uniform decrease in water potential of ~ -0.66 MPa at the turgor loss point across all populations. The Huber Value was higher at the end of the dry season, indicating a decrease in leaf area that could be supported by sapwood area. The hydraulic safety margin (difference between dry season Ψ_{MD} and $P_{50/88}$) was positive at all sites, with the smallest safety margin occurring for the second driest site at 700 mm MAP (Table **2**). However, trees at all other sites operated with a comparably high HSM of > 1 MPa for P_{50} and > 2 MPa for P_{88}.

Inherent genetic variation in functional traits - nursery experiment

The seedlings grown under uniform conditions demonstrated significant differences among the populations for $P_{50/88}$, LA, SLA, D_h and Wall (Table **S1**). Seedlings which originated from drier environments had significantly smaller leaves, a lower leaf hydraulic vulnerability, thicker walls between vessels and smaller hydraulic weighted vessel diameters (Fig. **2**) than seedlings from wetter environments. Additionally, the seedlings expressed a similar response to the trees studied in the field (Fig. **1**). The leaf size increased from 29.3 cm^2 per leaf for the dry-origin population to 39.9 cm^2 per leaf for the wet-origin population and was for all populations 40 to 70% larger than that measured in the field. Overall, the nursery saplings were also more hydraulically vulnerable than the mature field populations.

The dry-origin population BR had the lowest leaf hydraulic vulnerability with P_{50} = -3.30 MPa while the wet-origin population LH had the highest vulnerability (P_{50} = -2.33 MPa). The vessel walls were thickest in the dry-origin BR population with Wall = 2.82 µm^2. In contrast, seedlings expressed no significant trait variation among the populations for K_{max}, SLA, Ψ_{TLP} and HV. Except for these functional traits, all other studied functional traits differed among populations, indicating a genetic control over the trait expression.

Discussion

Trait variation along the precipitation gradient

We observed distinct differences in some functional traits along the precipitation gradient in the field. Four (Ψ_{MD}, Ψ_{TLP}, LA, P_{50}/P_{88}) of the 10 traits varied under well-watered winter (wet season) conditions along the precipitation gradient (Table **3**). Dry-origin populations showed higher drought tolerance, as indicated by lower Ψ_{TLP} (Bartlett *et al.*, 2014) and had a greater hydraulic safety margin by expressing a lower leaf hydraulic vulnerability (P_{50}/P_{88}). It is important to note that we measured leaf hydraulic vulnerability, and that due to hydraulic segmentation in *Eucalyptus* (Blackman *et al.*, 2019; Lucani *et al.*, 2019b) P_{50} derived from stems could yields different results. While we know that sapwood P_{50} adjust to some extend along climatic gradients (López *et al.*, 2013; Stojnić *et al.*, 2018) and that low sapwood hydraulic vulnerability reduces the risk of drought-induced tree mortality (Brodribb & Cochard, 2009; Urli *et al.*, 2013; Adams *et al.*, 2017), similar studies are still missing for the leaf hydraulic vulnerability. If leaves operate as 'hydraulic fuse' under drought to delay or prevent sapwood embolism (Tyree & Zimmermann, 2002; Rodriguez-Dominguez *et al.*, 2018), then their hydraulic vulnerability is critical as leaf cavitation will lead to tissue death and induce leaf shedding, which ultimately limits productivity. Although some researchers reported no intra-specific variation in sapwood vulnerability (Maherali & DeLucia, 2000), population studies in *Fagus sylvatica* L. (Stojnić *et al.*, 2018), *Quercus ilex* L. (Peguero-Pina *et al.*, 2014), *Pinus pinaster* Aiton (Corcuera *et al.*, 2011) and *Cordia alliodora* (Ruiz & Pav.) Oken (Choat *et al.*, 2007) confirm our findings of a lower hydraulic vulnerability in dry-origin populations, albeit on a different organ. It is possible that leaf hydraulic vulnerability is more plastic than sapwood vulnerability, as new leaves with a different anatomical structure (*e.g.* midrib vessel size and wall thickness, mesophyll tissue) could be generated depending on the environmental requirements. However, while evidence exist, that leaf midrib anatomy and conductance can adjust in

response to light (Scoffoni *et al.*, 2015), similar evidence for drought induced anatomical changes in the leaf midrib is still missing. A recent study on two *E. globulus* populations, observed indeed differences in leaf conductance in response to different growth habitats (Lucani *et al.*, 2019a). Leaf hydraulic conductance has been shown to decline with leaf vein embolism (Brodribb *et al.*, 2016) which suggests, that changes in the vein xylem rather than the outside xylem induce plastic changes in hydraulic conductance. However, no evidence of leaf xylem anatomical changes with drought exists to confirm this link and only the dry-origin population adjusted leaf conductance (Lucani *et al.*, 2019a), suggesting that changes in leaf hydraulic conductance are not uniform, even across populations. Trees from dry-origin populations also had smaller leaves which could be an adaptation to minimise water demand for transpiration or cooling and thereby increase hydraulic safety (Nardini *et al.*, 2014). Overall, the combined effect of lower Ψ_{TLP}, LA and P_{50}/P_{88} in the dry-origin populations contributes towards an increased hydraulic safety and increased drought resistance. We observed no trade-off in hydraulic efficiency for hydraulic safety among the populations. In fact, the results indicate that populations with low leaf hydraulic vulnerability also have an increased hydraulic conductivity (Fig. **S3**). While not significant, K_{max} declines (Fig. **1h**) and P_{50} significantly increases along the precipitation gradient (Fig. **1g**). This observation coincides with observations on over 300 species globally, where a substantial variability in K_{leaf} was observed for P_{50} values between -2 and -4 MPa, with some species being resistant to embolism (low P_{50}), despite having a high K_{leaf} (Gleason *et al.*, 2016; Pratt & Jacobsen, 2017). Overall, intra-specific trait variation was limited, and existing trait variation suggests higher hydraulic safety for dry-origin populations.

Short-term trait adjustment - seasonal trait variation

All *E. obliqua* populations demonstrated seasonal trait adjustments. Populations growing in drier environments experienced greater water deficits during the dry season (more negative Ψ_{PD} and Ψ_{MD}). In contrast with recent inter-specific studies (Johnson *et al.*, 2018), our results suggested no intra-specific differences in the plasticity of Ψ_{TLP} in relation to the experienced drought stress. Dry-origin populations experienced considerable seasonal changes in Ψ_{PD} and Ψ_{MD}, but lowered Ψ_{TLP} by a similar magnitude (0.66 ± 0.20 MPa) to wet-origin populations, which experienced less water stress. Nevertheless, dry-origin populations expressed the lowest Ψ_{TLP} during the wet and dry season. The plasticity in Ψ_{TLP} was caused by an increase in osmolytes (data not shown), likely to facilitate water uptake during the dry season (Mitchell *et al.*, 2008). Dry-origin populations also decreased SLA during the dry

season (Ramírez-Valiente *et al.*, 2017), potentially through selective retention of leaves (Ishii *et al.*, 2002). Leaf shedding resulted in an increase in HV during the dry season, with the population at the driest site showing the highest HV. This increase in HV is likely a direct response to drought stress and may reduce the tension on the water transporting system by reducing leaf area per unit sapwood area (Carter & White, 2009). The daily water use of trees is related to their overall leaf area (Hatton *et al.*, 1998) and one of the fastest ways to reduce water demand as drought stress increases is leaf shedding (Eagleson, 1982; Hasselquist *et al.*, 2010), which can be observed between seasons (Macinnis-Ng *et al.*, 2004), years (Pook, 1986) or along climatic gradients, where trees at drier sites support less leaf area per unit sapwood area (Mencuccini & Grace, 1995). While it is possible that other factors (*e.g.*, pests, herbivories) influenced the observed seasonal trait adjustment, phenotypic plasticity responding to environmental fluctuations in time (Chevin *et al.*, 2010), such as the change in the soil water availably with season is likely the main driver. Hence, phenotypic plasticity facilitated increased drought tolerance during the dry season to increase the overall hydraulic safety of trees whilst the wet season expression increased the hydraulic efficiency. The ability of traits to respond to short-term changes in the environment, *i.e.*, being phenotypically plastic, is likely to facilitate resilience under climate change (Lande, 2009).

Our results indicate that the increase of hydraulic safety and drought tolerance during the dry season takes place at different organ levels. At the leaf level, *E. obliqua* populations increased their hydraulic safety during the dry season by extending the range of Ψ under which the cells remained functional (lower Ψ_{TLP}), as well as by strengthening the leaves through an increased carbon investment (lower SLA in dry populations) into the non-vessel leaf tissue (Nardini *et al.*, 2012). Both Ψ_{TLP} and SLA contribute to hydraulic safety as well as efficiency. The contribution of Ψ_{TLP} to hydraulic efficiency is based on preventing conductivity loss by lowering and maintaining the cell turgor under drought (Trifiló *et al.*, 2016). Furthermore, the lowering of Ψ_{TLP} also triggers aquaporin deactivation (Yaaran & Moshelion, 2016), providing an increase in hydraulic safety. High SLA may increase leaf hydraulic conductance and therefore provides hydraulic efficiency benefits, while low SLA contributes to hydraulic safety as structural changes in leaf anatomy strengthen the leaf to withstand drought stress (Poorter *et al.*, 2009). In contrast to other studies (Metcalfe *et al.*, 1990; Xu *et al.*, 2009; McLean *et al.*, 2014), the leaf size in all *E. obliqua* populations remained unchanged, indicating that LA was not phenotypically plastic when exposed to seasonal changes in environmental parameters. We did not test for potential phenotypic

plasticity in the leaf xylem anatomy and leaf hydraulic vulnerability due to the logistical challenge of rehydrating the stressed branches in summer. However, several studies suggest that the hydraulic vulnerability (Van der Willigen & Pammenter, 1998; Lamy *et al.*, 2014) and xylem anatomy (dos Anjos *et al.*, 2015; Scoffoni *et al.*, 2015) express only minor amounts of phenotypic plasticity. Moreover, we did not observe plasticity in leaf xylem anatomy and leaf hydraulic vulnerability after exposing *E. obliqua* trees to either drought and/or well-watered conditions for nine months (Pritzkow, unpublished data). At the branch level, an increase in hydraulic safety arises through the reduction of leaf area per unit sapwood area (increase in HV through leaf shedding) which leads to an adjustment of the transpirational area to water availability (Merchant *et al.*, 2007; Rosas *et al.*, 2019). While this adjustment provides hydraulic safety benefits, the loss of overall leaf area also comes at the cost of reduced carbon gain, which could reduce non-structural carbohydrate reserves (Smith *et al.*, 2018) and increase the risk of carbon starvation. Conversely, a high leaf area per unit sapwood area (low HV) increases carbon gain and is highly efficient, as sapwood hydraulic efficiency may increase with decreasing HV (Tyree *et al.*, 1998; Gleason *et al.*, 2012; Gleason *et al.*, 2016). Thus, the combined plasticity at the leaf and branch level likely reduced the risk of hydraulic failure and therefore the risk of drought-induced mortality.

Inherent genetic variation in functional traits - nursery experiment

We detected similar trait expressions for the nursery-grown saplings compared to the trees in the field. Significant intra-specific differences were observed in the expression of LA, SLA, P_{50}/P_{88}, K_{max}, Wall and D_h (Table **S1**), indicating that expression of these traits is genetically controlled. These trait differences were also related to the climate of origin of the populations (Fig. **2** with the exception of SLA and K_{max}) and therefore likely reflected long-term environmental selection (Dutkowski & Potts, 2012). Similar observations have also recently been reported among *Eucalyptus* species (Bourne *et al.*, 2017; Li *et al.*, 2018). Dry-origin populations grew significantly smaller leaves with smaller vessels and thicker inter-vessel walls. Thicker inter-vessel walls and smaller vessel sizes as observed for dry-origin populations increase hydraulic safety, as the anatomical structure can withstand more negative pressures (Hacke *et al.*, 2001). The specific leaf xylem anatomy of dry-origin populations likely contributes in lowering P_{50} (Hacke *et al.*, 2006). However, K_{max} in dry-origin populations was not impaired by their anatomical structure as predicted by the Hagen–Poiseuille equation (Tyree & Zimmermann, 2002). Intra-specific differences in pit membrane porosity may counteract the effect of vessel size, as wide vessels can only achieve high

conductivity if their pit membranes are thin (Choat *et al.*, 2008; Hacke & Jansen, 2009). While the nursery seedlings also demonstrated no trade-off between P_{50} and K_{max}, as observed for other species (Meinzer *et al.*, 2009; Manzoni *et al.*, 2013), they differed from the mature field trees which tended to have high K_{max} combined with low P_{50}. This difference could be attributed to the different developmental stages or phenotypic plasticity in K_{max} depending on the environment. Overall, a suite of genetically controlled hydraulic traits facilitates higher drought tolerance for *E. obliqua* populations from drier environments, while other traits, such as Ψ_{TLP}, SLA, HV and K_{max}, indicate no inherent difference among *E. obliqua* populations.

Influence of genetics and phenotypic plasticity on hydraulic safety and efficiency

Within a species, populations can express intra-specific trait variation to facilitate an increased hydraulic safety or efficiency to enable their survival. These differences can also lead to different drought susceptibilities as shown for *E. obliqua*. We observed that dry-origin populations in the field expressed smaller leaves, with a lower hydraulic vulnerability and lower Ψ_{TLP} than their wet-origin counterparts, which likely increased their hydraulic safety and drought resistance. However, without knowing if the traits are driven by genetics and/or phenotypic plasticity, the capacity of populations to adjust with rapid climate change predictions remain uncertain. Here, the nursery saplings demonstrate, that all populations have a strong genetic control over key traits conferring hydraulic safety, whereas phenotypically plastic traits contributed, depending on their expression, to either hydraulic safety or efficiency. *E. obliqua* trees generated hydraulic safety through at least partly genetically determined traits, such as LA, $P_{50/88}$, D_h and wall thickness between adjacent vessels. While the literature indicates that phenotypic plasticity likely does not contribute, or only in a minor way in controlling the leaf anatomy and P_{50}/P_{88}, our setup does not allow a definite conclusion and future studies will be needed to investigate this further. However, while the nursery grown seedlings indicated a genetic control over the leaf anatomical trait expression, the leaf anatomy in the nature field populations demonstrates no response to their climate at origin. Hence, the results indicate that leaf anatomical traits are potentially partly under genetic and phenotypic plastic control which would enable some degree of phenotypic plasticity in the leaf conductivity. Nevertheless, other traits, such as Ψ_{TLP}, SLA and HV, showed no genetic influence and only seasonal trait adjustment, leading to the assumption that their trait expression at intra-specific level is driven by phenotypic plasticity. Phenotypically plastic traits provide a potentially increased hydraulic efficiency under

favourable environmental conditions and an increased hydraulic safety when conditions become unfavourable. Moreover, with rapid climate change and many functional traits conferring hydraulic safety being to some extent genetically regulated, the avoidance of drought-induced tree mortality might become extremely challenging. In particular, a strong genetic control over the leaf hydraulic vulnerability raises concerns, as this parameter was identified as a driving factor for tree distribution (Brodribb et al., 2019) and survival under climate change (Brodribb & Cochard, 2009; Choat et al., 2012). It seems unlikely that *E. obliqua* will be able to decrease hydraulic vulnerability in pace with climate change, given the generation cycles in long-lived organisms such as trees (Allen *et al.*, 2010). Hence, the importance of phenotypic plasticity for survival will increase under climate change, as genetically fixed, non-plastic traits could become a barrier. Plastic HV adjustments through leaf shedding are a highly effective way to reduce water deficit under increasing future drought situations and could help bridging the time for the in parts genetically fixed P_{50}/P_{88} to adapt to new conditions. Our results indicate that climate warming is likely to have similar effects across the studied *E. obliqua* populations, and by extension affect all *E. obliqua* populations growing under these environmental conditions similarly.

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Authors' contributions

SA, CS, VW and CP conceived the ideas and designed the study; CP collected and CP and RT analysed the data; all authors contributed to the writing which was led by CP.

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Figure legends

Figure 1: Seasonal changes in the relationship between mean annual precipitation at the origin of the *Eucalyptus obliqua* populations and a) pre-dawn water potential, b) midday water potential, c) water potential at turgor loss point, d) leaf size, e) Huber Value, f) specific leaf area, g) water potential at 50% and 88% loss of hydraulic conductivity, h) maximum hydraulic conductivity, i) hydraulic weighted vessel diameter and j) double wall thickness. For each season, tree mean trait values (circles, except for P_{50} and P_{88} where circles represent population mean), linear regressions (wet season: black line and dry season: grey line), correlation coefficients (r), with significance level indicated as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns $P > 0.05$ and 95% confidence interval are given.

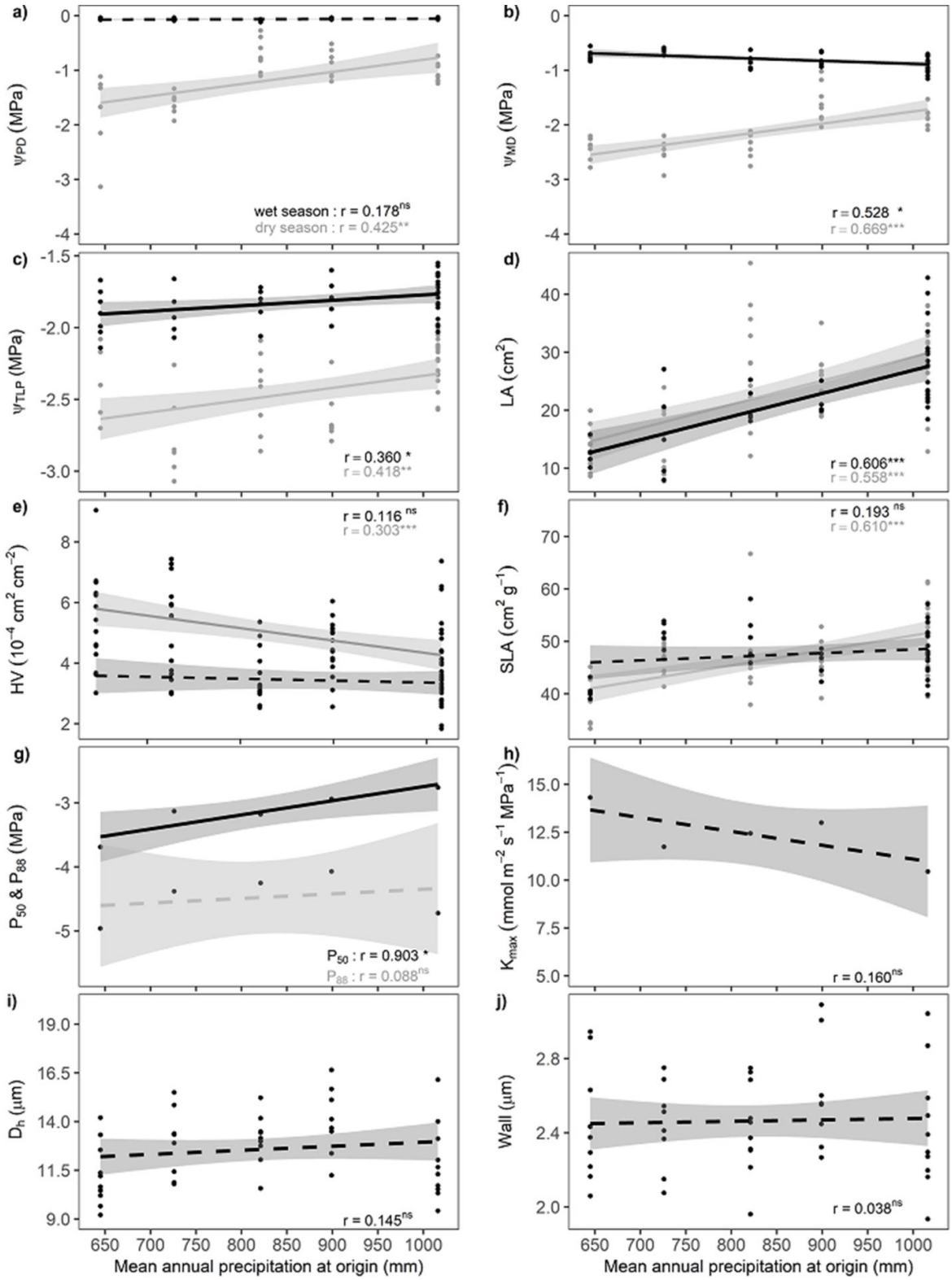
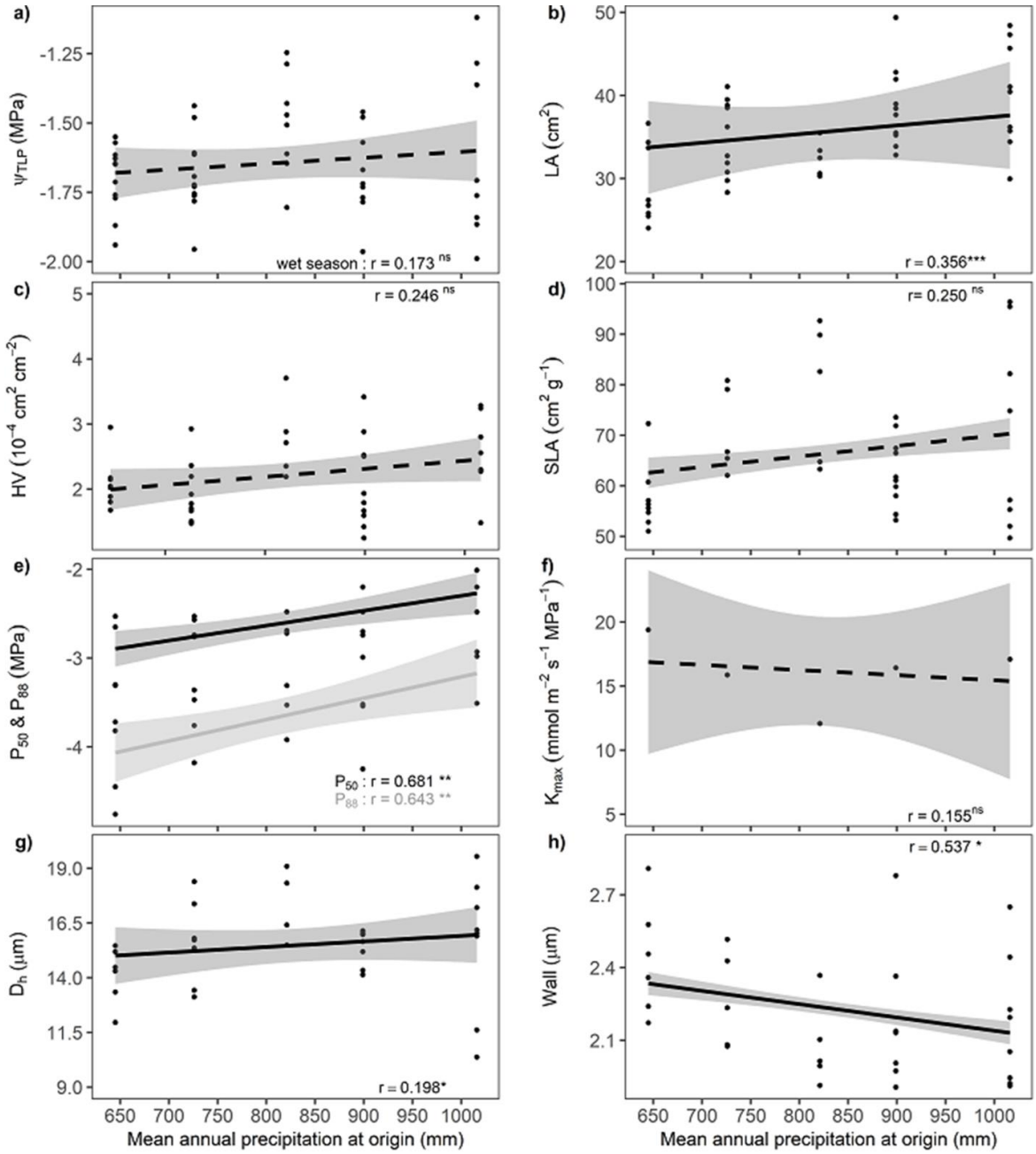

a)
ψ_{PD} (MPa)
wet season : r = 0.178^{ns}
dry season : r = 0.425**
b)
ψ_{MD} (MPa)
r = 0.528 *
r = 0.669***
c)
ψ_{TLP} (MPa)
r = 0.360 *
r = 0.418**
d)
LA (cm^2)
r = 0.606***
r = 0.558***
e)
HV (10^{-4} cm^2 cm^{-2})
r = 0.116 ^{ns}
r = 0.303***
f)
SLA (cm^2 g^{-1})
r = 0.193 ^{ns}
r = 0.610***
g)
P_{50} & P_{88} (MPa)
P_{50} : r = 0.903 *
P_{88} : r = 0.088^{ns}
h)
K_{max} (mmol m^{-2} s^{-1} MPa^{-1})
r = 0.160^{ns}
i)
D_h (µm)
r = 0.145^{ns}
j)
Wall (µm)
r = 0.038^{ns}
Mean annual precipitation at origin (mm)
Mean annual precipitation at origin (mm)

Figure 2: Relationship between mean annual precipitation at origin and functional traits of 8-months old seedlings grown from five *Eucalyptus obliqua* populations from seed along a precipitation gradient. Tree mean trait values during the wet season (circles) for a) water potential at turgor loss point, b) leaf size, c) Huber Value, d) specific leaf area, e) water potential at 50% and 88% loss hydraulic conductivity, f) maximum hydraulic conductivity, g) hydraulic weighted vessel diameter and h) double wall thickness, linear regressions with an 95% confidence interval, correlation coefficients (r) and corresponding p-values are given.



Tables

Table 1: List of physiological, morphological and anatomical traits (measured in the leaf midrib), including their units, definitions, acronyms and association with hydraulic efficiency (efficiency), hydraulic safety (safety) or both, as described in the cited literature.

Trait	Unit	Description / Formula	Hydraulic implications
Physiology			
Ψ_{PD}	MPa	Pre-dawn water potential	-
Ψ_{MD}	MPa	Midday water potential	-
Ψ_{TLP}	MPa	Water potential at turgor loss point	Efficiency [a] & Safety [b]
$P_{50/88}$	MPa	Water potential at 50% or 88% loss of ctivity	Safety [c]
K_{max}	mmol m^{-2} s^{-1} MPa^{-1}	Maximum hydraulic conductivity	Efficiency [k]
HSM_X	MPa	Hydraulic safety margin (Ψ_{MD} - P_{50} or Ψ_{MD}	Safety [d]
Morphology			
LA	cm^2	Leaf size	Safety [e]
SLA	cm^2 g^{-1}	Specific leaf area	Efficiency [e] & Safety [f]
HV	10^{-4} cm^2 cm^{-2}	Huber Value; HV = sapwood area/ leaf	Efficiency [g] & Safety [h]
Leaf anatomy			
Wall	µm	Double wall thickness between adjacent	Safety [i]
D_h	µm	Hydraulic weighted vessel diameter	Efficiency [m] & Safety [n]

Reference: [a] Trifiló et al. (2016); [b] Yaaran & Moshelion (2016); [c] Nardini et al. (2013); [d] Anderegg et al. (2016); [e] Nardini et al. (2014); [f] Poorter et al. (2009); [g] Gleason et al. (2016); [h] Carter & White (2009); [i] Guet et al. (2015); [k] Tyree & Zimmermann (2002); [m] Schuldt et al. (2013); [n] Larter et al. (2017)

Table 2: Location, mean annual precipitation (MAP), mean annual temperature (MAT) and Heat Moisture Index (HMI) for the five *Eucalyptus obliqua* field sites and Melbourne, the site we conducted the nursery experiment. The HSM_{50} and HSM_{88} refer to the hydraulic safety margin between the water potential at 50% or 88% loss of leaf hydraulic conductivity (P_{50} or P_{88}) and dry seasc minimum water potential (Ψ_{MD}). Mean annual climate data were derived using ANUCLIM v6 (beta) with 0.01 degree resolution and centred o 990 (1976-2005).

Location	Site code	Latitude (˚N)	Longitude (˚E)	Elevation (m a.s.l.)	MAP (mm)	MAT (°C)	HMI	HSM_{50} (MPa)	HSM_{88} (MPa)
Population:									
Brisbane Ranges	BR	-37.868	144.218	311	638	13.0	36.0	1.10	2.48
Mount Egerton	ME	-37.620	144.157	520	726	12.0	30.3	0.59	1.78
Daylesford	DF	-37.359	144.119	536	822	11.5	26.2	1.02	2.32
Wombat	WO	-37.406	144.126	633	899	11.0	23.4	1.10	2.74
Leonards Hill	LH	-37.418	144.181	778	1016	10.4	20.1	1.42	3.84
Nursery experiment:									
Melbourne	MB	-37.829	145.023	10	667	14.8	37.2		

Table 3: GLMM results for the effects of mean annual precipitation (MAP) at the origin of the *Eucalyptus obliqua* populations, season and the interaction between MAP x season on functional traits with associated probability (P; *** P < 0.001; ** P < 0.01; * P < 0.05; ns P > 0.05) and degrees of freedom (DF). The intercept coefficients represent the log transformed mean trait value (coef and standard error (SE) during the wet season, and $MAP_{wet\ season}$ and $MAP_{dry\ season}$ coefficients represent the slope of each trait with MAP (e.g. relation of trait expression to MAP) during the wet and dry season, respectively. The Season coefficient represents the trait difference betwe the wet and dry season (e.g. phenotypic plasticity), and the MAP x season interaction represents the MAP slope difference between wet and dry season (e.g. differences in the magnitude of phenotypic plasticity between populations).

Traits	*Intercept*	*$MAP_{wet\ season}$*		*$MAP_{dry\ season}$*		*Season*			*MAP x Season*		*DF*
	coeff (SE)	*coeff (SE)*	*P*	*coeff (SE)*	*P*	*coeff (SE)*	*P*		*coeff (SE)*	*P*	
Ψ_{PD}	-2.802 (0.05)	-0.069 (0.05)	0.142 ^{ns}	-0.220 (0.06)	0.006 **	2.838 (0.09)	0.0(	**	0.151 (0.08)	0.086 ^{ns}	75
Ψ_{MD}	-0.242 (0.02)	-0.093 (0.02)	0.000***	-0.150 (0.03)	0.000***	0.957 (0.04)	0.0(	**	0.243 (0.04)	0.000***	82
Ψ_{TLP}	0.595 (0.01)	-0.053 (0.01)	0.019*	-0.048 (0.01)	0.001***	0.291 (0.01)	0.0(	**	0.019 (0.02)	0.337 ^{ns}	86
LA	2.946 (0.04)	0.299 (0.04)	0.000***	0.298 (0.04)	0.000***	0.098 (0.06)	0.1	^{s}	0.001 (0.06)	0.984 ^{ns}	92
SLA	3.856 (0.02)	0.023 (0.02)	0.178 ^{ns}	0.088 (0.03)	0.000***	-0.013 (0.05)	0.5	^{s}	-0.065 (0.05)	0.003 **	92
HV	1.161 (0.04)	-0.044 (0.04)	0.262 ^{ns}	-0.110 (0.01)	0.001***	0.360 (0.02)	0.0(	**	0.067 (0.02)	0.183 ^{ns}	92
P_{50}	1.139 (0.02)	-0.101 (0.02)	0.023*	-	-	-	-		-	-	3
P_{88}	1.496 (0.04)	-0.023 (0.04)	0.646 ^{ns}	-	-	-	-		-	-	3
K_{max}	2.511 (0.04)	-0.085 (0.05)	0.160 ^{ns}	-	-	-	-		-	-	3
Wall	0.888 (0.02)	0.008 (0.02)	0.650 ^{ns}	-	-	-	-		-	-	46
D_h	2.525 (0.04)	0.023 (0.04)	0.639 ^{ns}	-	-	-	-		-	-	3

Traits: Ψ_{PD}: pre-dawn water potential, Ψ_{MD}: midday water potential, Ψ_{TLP}: water potential at turgor loss point, LA: leaf area, SLA: spec leaf area, HV: Huber Value, P_{50}: loss of 50% hydraulic conductivity, P_{88}: loss of 88% hydraulic conductivity, K_{max}: maximum hydraulic conductivity, Wall: double wall thickness, D_h: h aulic weighted vessel diameter